



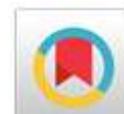
## ***In vitro* inhibition of uropathogenic *Escherichia coli* biofilm formation by probiotic Lactobacilli isolated from healthy breast fed infants**

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### **Abstract**

Biofilm forming *Escherichia coli* bacterium exhibits multiple drug resistance, which is responsible for recurrent urinary tract infections (UTI) that are difficult to eradicate. The work aimed to investigate the antibacterial and antibiofilm activities of Lactobacilli isolated from fecal microbiota of healthy infants; to identify these isolates and determine their probiotic characteristics. *E. coli* isolates were recovered from urine samples of patients with urinary tract infections. On the other hand, *Lactobacillus* isolates were recovered from feces of breast-fed infants. Five strong biofilm forming *E. coli* isolates with multidrug resistance (MDR) were selected. The antibacterial potential of *Lactobacillus* supernatants were assessed via disk diffusion assay. All the tested *E. coli* isolates showed high susceptibility to the *Lactobacillus* supernatants; where 54 % of these supernatants expressed inhibition zones diameters ranging from 15- 18 mm. Antibiofilm efficacies of *Lactobacillus* spp. against *E. coli* isolates were tested *in vitro* using microtiter plate assay and scanning electron microscopy (SEM). More than 50 % reduction of biofilms formation by the 5 selected MDR *E. coli* isolates was observed by most the *Lactobacillus* isolates. The scanning electron microscopy confirmed the elimination of *E. coli* biofilms by cells of the *Lactobacillus* isolates. Preliminary probiotic characteristics of the *Lactobacillus* isolates were investigated; all isolates tolerated 2 % bile salt concentration and acidic condition at pH 3. Regarding safety of the Lactobacilli for human consumption, all isolates were non hemolytic, and 14 *Lactobacillus* isolates were sensitive to all tested antibiotics except for vancomycin, as they are naturally resistant to it. About 14 safe probiotic *Lactobacillus* isolates were identified by API-50 CHL test as; *Lactobacillus acidophilus*, *L. plantarum*, *L. fermentum* and *L. paracasei*.

**Keywords:** *Lactobacillus* spp., Probiotic, Antibacterial activity, Multidrug resistance, Biofilm formation

### **1. Introduction**

Urinary tract infection (UTI) is among the common microbial infections that affect 150 million individuals around the world every year. A previous

study conducted by [Toval et al., \(2014\)](#) reported that uropathogenic *E. coli* (UPEC) infrequently causes nosocomial UTIs (50 %) and community-acquired

infections (90%). These bacterial uropathogens harbor various virulence determinants essential for initial adhesion and colonization of the mucosal surfaces; cell and tissue invasion, avoiding the host defence mechanisms, and causing persistent chronic infections. These virulence determinants include surface factors as fimbriae and extracellular factors such as toxins and polysaccharide coatings, as highlighted by [Emody et al., \(2003\)](#). According to the research work of [Eberly et al., \(2017\)](#), UPEC develops biofilms on the outside surface of the urethral catheters, in addition to urinary bladder mucosa. Biofilm microbes are more resistant to treatment with antibiotics than plankton ([Donlan, 2001](#)). As reported recently by [Aslam et al., \(2018\)](#), the increase of antibiotic resistance rate has encouraged researchers to search for antimicrobial alternatives, of which probiotics have gained increasing interests. Probiotics are “live bacteria which when administered in sufficient amounts confer a health benefit on the host” ([FAO/WHO, 2002](#)). Probiotics comprise beneficial bacteria, of which the *Lactobacillus* and *Bifidobacterium* genera are the most common studied types. These bacteria produce antimicrobial secondary metabolites. Lactobacilli are generally regarded as safe (GRAS) bacteria, and therefore could be used safely as probiotics for several medical applications ([Wadoum et al., 2019](#)). New strategies are being attempted by researchers to manage urinary catheter biofilms, such as the coating of catheters with bio-surfactants, probiotics and other non-pathogenic microorganisms, as revealed by previous studies of [Borchert et al., \(2008\)](#); [Sambanthamoorthy et al., \(2014\)](#).

Lactobacilli comprise about 0.01 % of the total microbiome in the gastrointestinal tract (GIT), and range between  $10^7$ -  $10^8$  cells/ g of human faeces ([Rinttilä et al., 2004](#)). Later, [Lebeer et al., \(2008\)](#) added that they possess health-promoting effects in GIT such as maintaining the normal intestinal homeostasis, by inhibiting its colonization by pathogenic bacteria and modifying the immune responses. [Mirlohi et al., \(2008\)](#) revealed that the physiological state of the new borne and infants, the

ecological differences and their genetics are considered as the major parameters affecting their Lactoflora. [Ahrné et al., \(2005\)](#) reported that changes in infant's age up to 6 months can cause significant changes in the enteric Lactobacilli. These changes may be correlated to the introduction of solid foods to infants' diet. The objectives of this study were to recover *Lactobacillus* isolates from the faecal microbiota of healthy breast fed new born, and studying their antibacterial and antibiofilm activity against multidrug resistant (MDR) *E. coli* strains. In addition to investigating the different criteria used for selection of the potent antibiofilm and safe probiotic isolates.

## 2. Material and methods

### 2.1. Culture and identification of bacterial isolates

About 50 stool samples were collected from healthy breast-fed infants (aged 3-6 months) from Children Hospital, Minia, Egypt. Ethical approvals were obtained from the Institutional Review Boards of the hospital. Standard 10-fold dilutions of the stool samples were made in 0.9 % sterile physiological saline and then each sample was plated on deMan Rogosa Sharp (MRS) selective agar medium. After incubation for 48 h, the obtained bacterial colonies were identified using biochemical assays, in reference to [Kılıç and Karahan, \(2010\)](#). *Escherichia coli* isolates were recovered from 50 urine samples of patients suffering from UTI at Minia University Hospital, after growth on the selective MacConkey and Eosin Methylene Blue agar media. The recovered *E. coli* isolates were identified biochemically, according to [Cheesbrough, \(1981\)](#).

### 2.2. Antibacterial susceptibility of the *E. coli* isolates

Antibacterial susceptibility assay was performed on Mueller-Hinton agar (MHA) (LAB M, UK) using Kirby-Bauer disk diffusion test according to [Hudzicki, \(2009\)](#), following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) ([Wayne, 2018](#)). The *E. coli* isolates were tested for sensitivity to

several antibiotics purchased from Bio-analyse R, Turkey. *E. coli* isolates were considered MDR, if they showed resistance to three or more antibiotics belonging to different antibiotics classes or groups.

### 2.3. Biofilm formation by *E. coli* isolates

Biofilm formation was tested using 96-well polystyrene microtiter plates, following the method reported by [Stepanović et al., \(2007\)](#). Overnight cultures of *E. coli* were diluted to 0.5 McFarland using Mueller-Hinton broth (MHB), and then 200 µl of each of the diluted suspensions was pipetted individually into the wells of microtiter plate. The plates were incubated at 37°C for 48 h, using sterile MHB as a negative control. After incubation, the planktons were removed and the attached cells were fixed for 15 min. using 200 µl of methanol (99 %). The methanol was discarded and then 200 µl of crystal violet (2% w/v) was added to each well, followed by incubating the plates for 15 min. at room temperature. Finally, 200 µl of 33 % (w/v) acetic acid was added to each well, and the optical density OD<sub>540</sub> was measured using a micro ELISA auto reader (model 680, Bio rad). Based on the obtained OD, the isolates were classified as biofilm non-producers (OD ≤ OD<sub>c</sub>), weak producers (OD<sub>c</sub> < OD ≤ 2OD<sub>c</sub>), moderate producers (2OD<sub>c</sub> < OD ≤ 4OD<sub>c</sub>) and strong producers (4OD<sub>c</sub> < OD). Tests were performed in triplicates and repeated twice; where OD<sub>c</sub> is the optical density of negative control, and OD is the optical density of the tested *E. coli* isolates.

### 2.4. Antibacterial potential of *Lactobacillus* supernatants

For preparation of the *Lactobacillus* cell-free supernatant (CFS), isolates were cultured in MRS broth at 37°C for 18 h. After incubation, broths were centrifuged at 10,000× g for 5 min., and then the supernatants were stored at -20°C until use ([Aly et al., 2018](#)). The antibacterial efficacy of these CFSs against the selected *E. coli* isolates was determined using the well diffusion method, according to [Davoodabadi et al., \(2015a\)](#). For each *E. coli* isolate, 100 µl of 24 h cell suspension (0.5 McFarland turbidity) was spread

onto the surface of MHA plate using a sterile glass spreader, and then 6 mm wells were punched in the media using a sterile cork borer. An aliquot of 100 µl of CFSs was poured individually into each well, and then the plates were incubated at 37°C for 24 h. After incubation the inhibition zones were measured using a calibrated ruler. Three replicates were used for each CFS and the test was repeated thrice.

### 2.5. Effect of *Lactobacillus* supernatants on the preformed bacterial biofilms

The effects of the potent Lactobacilli CFSs on the dispersion of biofilms formed by the MDR *E. coli* isolates were determined, as described in the recent study conducted by [Kaur et al., \(2018\)](#). The overnight culture of each *E. coli* isolate was adjusted to 0.5 McFarland using MHB. An aliquot of 100 µl of the diluted cultures was added to each well in 96-well microtiter plates, and then incubated at 37°C for 48 h. After incubation, the free cells were removed and then washed gently 3 times with sterile saline. Lactobacilli CFSs (100 µl) were added individually to the wells. In the control wells, 100 µl of sterile MRS broth was added instead of CFS. The plates were incubated at 37°C for 24 h. The experiment was conducted in triplicates. The biofilm formation was quantified using crystal violet method as described above by [Stepanović et al., \(2007\)](#). The percentage (%) reduction in biofilm formation was evaluated using the following equation of [Abdelhamid et al., \(2018\)](#):

$$\text{Percentage of inhibition (\%)} = 100 - \left( \frac{\text{OD}_{540} \text{ of test wells}}{\text{OD}_{540} \text{ of control well}} \right) \times 100$$

### 2.6. Scanning electron microscopy

The scanning electron microscopy (SEM) was used to detect changes in the structures of biofilms, caused by the interactions between cells of the *Lactobacillus* isolates and the selected MDR *E. coli* isolate. Overnight MRS cultures of *Lactobacillus* and MHB culture of *E. coli* isolate were diluted (0.5 McFarland), and then mixed in equal volumes. Biofilms were allowed to form in 6 wells polystyrene microtiter plates and then incubated for 48 h. The non-

adherent cells were removed by rinsing with phosphate buffered saline (PBS) 3 times. Biofilms were then fixed according to [Wu et al., \(2015\)](#), and then prepared for examination by JSM-840 SEM (JEOL Ltd., Tokyo, Japan).

## 2.7. Sensitivity of the *Lactobacillus* isolates to bile salts and tolerance to acidic pH

Sensitivity to bile salts was assessed by culturing the *Lactobacillus* isolates on MRS agar with 2 % (w/v) bile salt (Merck), and then the cultures were incubated for 48 h at 37°C. For testing tolerance of Lactobacilli to an acidic pH (3.0), the overnight cultures were centrifuged at 6000 ×g at 4°C for 15 min. The pellets were re-suspended in the same volume of 0.9% (w/v) saline adjusted at pH 3.0. Suspensions were incubated at 37°C for 3 h, and then centrifuged. The formed pellets were cultured on MRS plates and then incubated at 37°C for 48 h, as described by [Nouri et al., \(2010\)](#). Each experiment was repeated twice and conducted in triplicates.

## 2.8. Detection of safety of the *Lactobacillus* isolates for application in the treatment of human UTI

### 2.8.1. Potency of blood hemolysis

According to [Kacem and Kaid-Harche, \(2008\)](#), the Lactobacilli isolates were cultured overnight in MRS broth. Cultures were streaked on blood agar plate (LAB M, UK), and then plates were incubated at 37 °C for 24 h. After incubation, these plates were inspected to detect the presence of; clear, greenish, or no lysis zones, which indicated the presence of β-hemolytic, α-hemolytic and γ-hemolytic isolates, respectively.

### 2.8.2. Antibiotic susceptibility of the *Lactobacillus* isolates

The disk diffusion method of [Hudzicki, \(2009\)](#) was carried out to test for the antibiotic susceptibility of the *Lactobacillus* isolates. The isolates were adjusted to 0.5 McFarland using MRS broth. The used antibiotic disks were obtained from Bio-analyse R,

Turkey. After incubating the plates at 37°C for 24 h, the formed inhibition zone diameters were measured using a calibrated ruler, according to the criteria of CLSI guidelines in reference to [Wayne, \(2018\)](#). The experiment was repeated twice and done in triplicates.

## 2.9. Identification of *Lactobacillus* isolates to the species level

The API-50 CHL test (Biomereux, France) was used for identification of the potent antibiofilm *Lactobacillus* isolates, according to the manufacturer's instructions. The fermentation profiles were recorded, and the Biomereux database (France) was used for interpretation of the results, in reference to [Ozgun and Vural, \(2011\)](#).

## 2.10. Statistical analysis

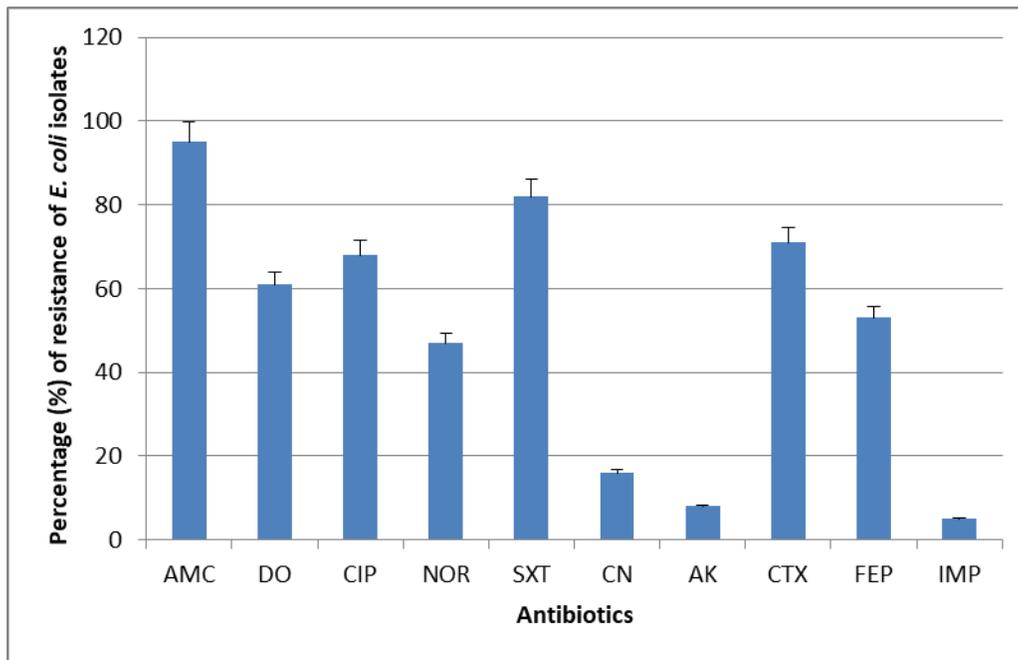
Statistical analyses of results were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL). The antibiofilm activity of *Lactobacillus* CSFs was analysed by One Way Analysis of Variance (ANOVA) Tukey's multiple-comparison test. P<0.05 indicates significant difference.

## 3. Results

In the current study, about 41 isolates were obtained from the 50 stool samples (82 %) and then identified biochemically as *Lactobacillus* sp., whereas 38 isolates were recovered from the 50 urine samples (76 %) and identified as *E. coli*.

### 3.1. Antibiotic sensitivity and biofilm formation by the *E. coli* isolates

Antibiotic resistance of *E. coli* isolates was tested using disk diffusion method. High resistance to amoxicillin-clavulanic acid (95 %), trimethoprim/sulfamethoxazole (82 %), cefotaxime (71 %) and ciprofloxacin (68 %) were recorded, and demonstrated in Fig. (1). In the microtiter plate assay, 13 (34 %), 8 (21 %) and 6 (16 %) of *E. coli* isolates demonstrated strong, moderate and weak biofilm formation abilities; respectively, whereas 11 (29%) were unable to form biofilm.



**Fig. 1.** Antibiotic resistance patterns of the 38 uropathogenic *E. coli* isolates. Results are averages of three replicate plates. Error bars represent standard deviations. Where; AMC: amoxycillin/ clavulanic acid, DO: doxycycline, CIP: ciprofloxacin, NOR: norfloxacin, SXT: trimethoprim/ sulfamethoxazole, CN: gentamicin, AK: amikacin, CTX: cefotaxime, FEP: cefepime and IMP: imipenem

About 5 MDR *E. coli* isolates out of the 38 mainly referred as; E5, E12, E17, E23, and E36, were selected to test the antibacterial and antibiofilm potentials of the *Lactobacillus* isolates. These isolates were resistant to 5 or more antibiotics belonging to different

antibiotic groups. At the same time, they showed strong biofilm formation capabilities. The antibiotic resistance pattern of these 5 selected *E. coli* isolates is shown in Table (1).

**Table 1.** Antibiotic resistance pattern of the MDR and strong biofilm forming 5 *E. coli* isolates

<i>E. coli</i> isolates	Antibiotics									
	AMC	DO	CIP	NOR	SXT	CN	AK	CTX	FEP	IMP
E5	R	R	R	R	R	R	S	R	S	S
E12	R	R	R	R	R	S	S	R	R	S
E17	R	R	R	R	R	R	R	R	R	S
E23	R	R	R	R	R	R	S	R	R	S
E36	S	R	R	R	R	R	R	R	S	R

Results are averages of three replicate plates. Where; S: sensitive, R: resistant, I: intermediate. AMC: amoxycillin/ clavulanic acid, DO: doxycycline, CIP: ciprofloxacin, NOR: norfloxacin, SXT: trimethoprim/ sulfamethoxazole, CN: gentamicin, AK: amikacin, CTX: cefotaxime, FEP: cefepime and IMP: imipenem

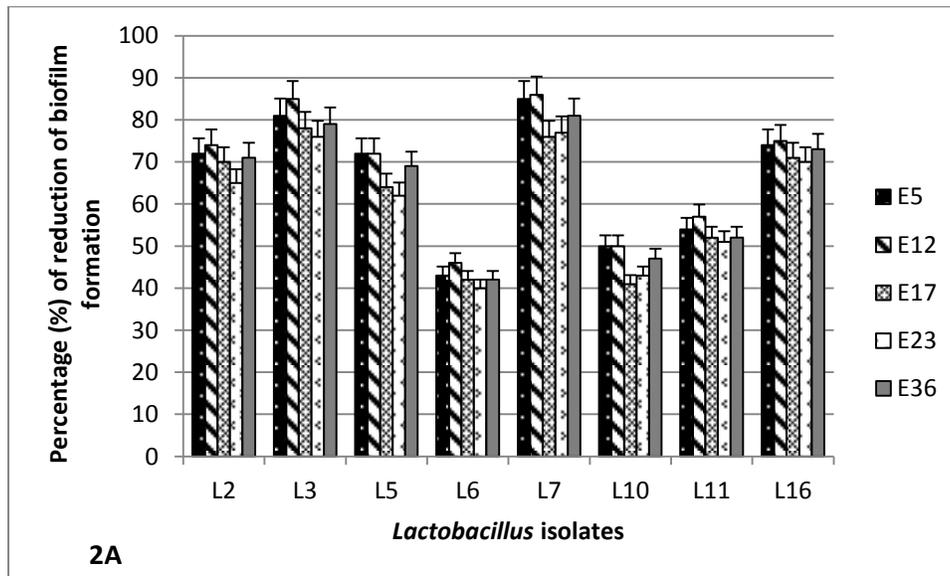
**3.2. Antibacterial potentials of Lactobacilli CFSs against *E. coli* isolates**

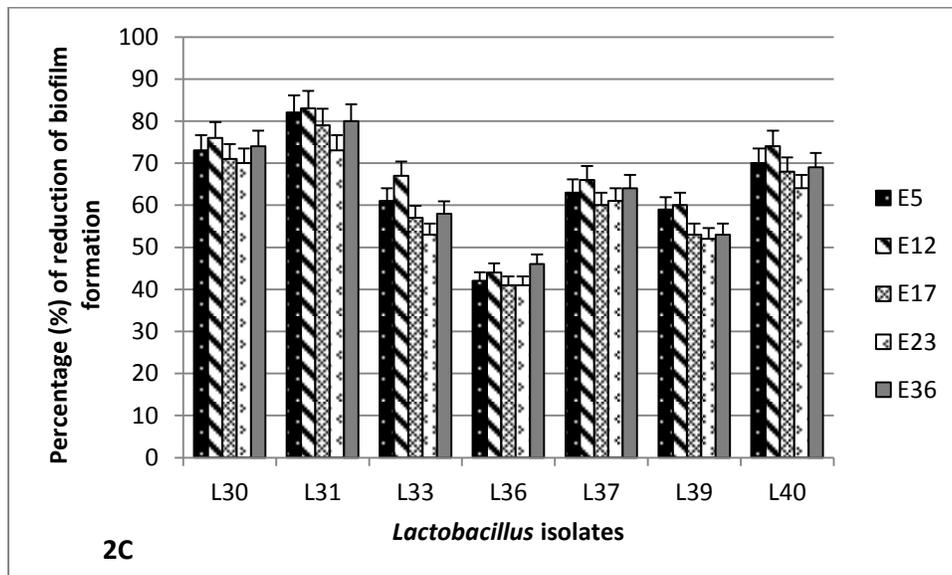
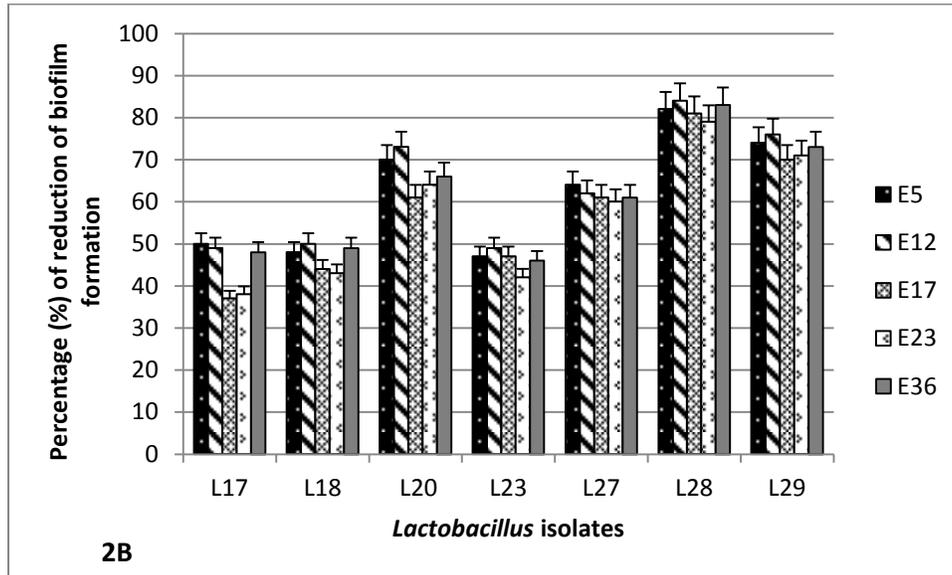
Antibacterial efficacies of 41 Lactobacilli supernatants against the 5 MDR *E. coli* isolates were assessed using agar well diffusion assay. The *E. coli* isolates were sensitive to all the Lactobacilli CFSs with an average inhibition zone diameters  $\geq 9$  mm. About 22 (54 %) out of 41 CFSs of the tested Lactobacilli isolates demonstrated highest inhibitory effects against the concerned *E. coli* isolates, exhibiting inhibition zones diameters ranging from 15-18 mm. The antibiofilm action of these selected 22 Lactobacilli CFSs was assessed.

**3.3. Effect of selected Lactobacilli CFSs on dispersion of the preformed biofilms**

Cell-free supernatant (CFS) of all the selected 22 *Lactobacillus* isolates expressed significant reductions of biofilm formation by the 5 MDR *E. coli* isolates, as demonstrated in Fig. 2(A, B and C).

Out of 22 CFSs, 16 caused more than 50 % reduction of biofilm formation; however, maximum inhibition was observed by isolate L28, giving reduction of 79 % - 85 % against the 5 tested *E. coli* isolates.



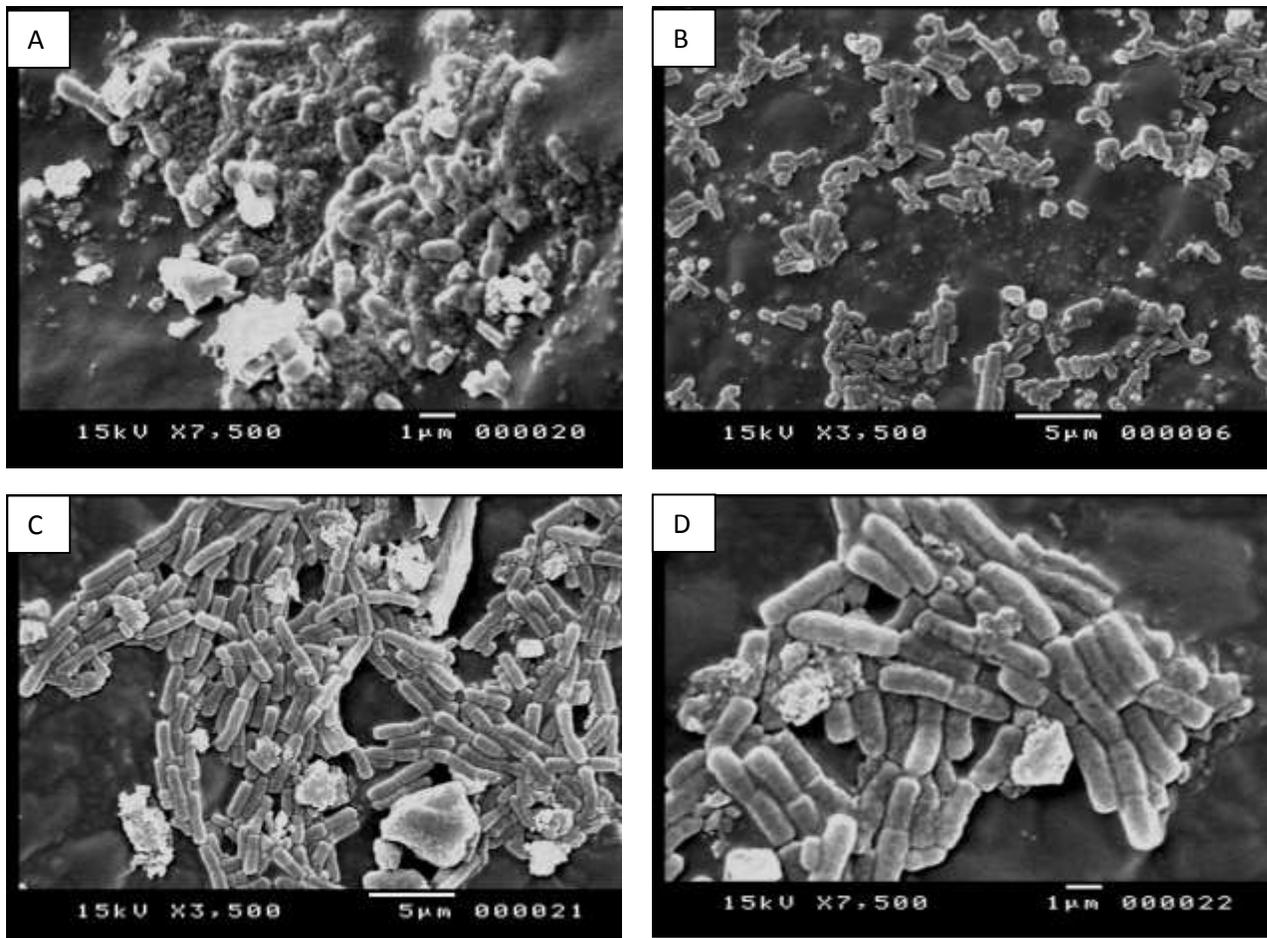


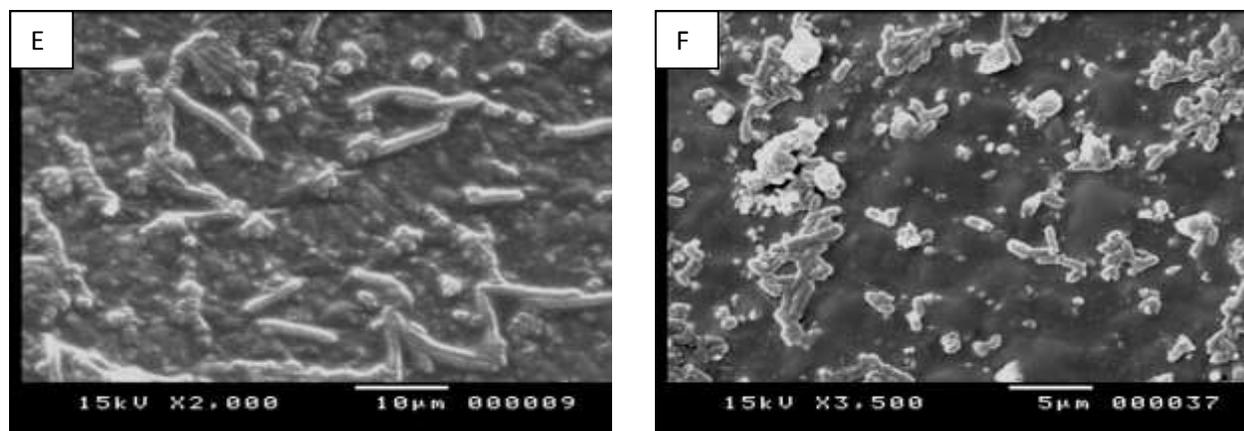
**Fig. 2 (A, B and C):** Activity of the 22 selected *Lactobacillus* CFSs isolates against biofilm formation by the 5 MDR *E. coli* isolates. Results are averages of three replicate plates. Where; E (5, 2, 17, 23 and 36) represent the selected MDR *E. coli* isolates

### 3.4. Scanning Electron micrographs

The effects of cells of some *Lactobacillus* isolates on the structure of biofilm formed by the E17 isolate (selected for being strong biofilm former and resistant to all tested antibiotics except one) upon co-culturing, was investigated using SEM. The *Lactobacilli* isolates assigned as; L3, L7, L28, L30 and L31, expressed the highest antibiofilm activities. *Lactobacillus* cells or its biofilms could replace the *E. coli* cells in their biofilms. Fig. (3 A) shows a dense biofilm mass

formed by E17. Co-culture of *Lactobacilli* isolates L3 and L31 with E17 (Fig. 3 B and F) showed fewer *E. coli* cells attached to the surface, with no dense aggregates. Elimination of *E. coli* biofilm formation and appearance of *Lactobacilli* aggregates of both L7 and L28 strains are demonstrated in Fig. (3 C and D). Co-inoculation of *Lactobacillus* isolate L30 with E17, showed *Lactobacillus* cells with decreased number of *E. coli* cells in the culture, as demonstrated in Fig. (3E).





**Fig. 3.** SEM images of *E. coli* isolate (17) as control (A), on co-culture with cells of *Lactobacillus* isolates; isolate L3 (B), isolate L7(C), isolate L28 (D), isolate L30 (E), and with isolate L31 (F)

### 3.5. Sensitivity of the *Lactobacillus* isolates to bile salts and acidic pH

All the 22 *Lactobacillus* isolates were able to grow in 2 % w/v concentration of bile salts, and at acidic pH (3).

### 3.6. Safety of the *Lactobacillus* isolates

The *Lactobacillus* isolates showed no hemolysis when cultured on blood agar plates. The antibiotic susceptibilities of Lactobacilli were determined. All *Lactobacillus* isolates were highly sensitive to tetracycline, chloramphenicol, and clindamycin. However, all isolates had intrinsic resistance to vancomycin. Out of the tested 22 *Lactobacillus* isolates; 1 (5 %), 2 (9 %), 5 (23 %), 5 (23 %) were resistant to cefazolin, erythromycin, gentamicin and ciprofloxacin; respectively, as shown in Table (2).

### 3.7. Identification of the *Lactobacillus* isolates

A total of 14 *Lactobacillus* isolates that were sensitive to all the tested antibiotics (except vancomycin), with acid and bile tolerance, non-hemolytic on blood agar, and currently expressed good antibacterial and antibiofilm activities were identified to the species level. Based on API-50 CHL identification, *Lactobacillus* isolates are identified as;

*Lactobacillus acidophilus* (L3, 5, 7, 11, 31 and 39), *L. plantarum* (L20, 27, 36 and 37), *L. fermentum* (L10, 16 and 28) and *L. paracasei* (L17).

## 4. Discussion

The rate of antibiotic resistance in uropathogenic *E. coli* (UPEC) that is the major causative agents of UTIs have increased and become difficult to manage, as highlighted recently by [Ramírez-Castillo et al., \(2018\)](#). In this study, 95 %, 82 %, 71 % and 68 % of the *E. coli* isolates demonstrated resistance to amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole, cefotaxime and ciprofloxacin, respectively.

In consistence with current results, previous study by [Ali et al., \(2016\)](#) reported resistance more than 70 % among the isolated UPEC to trimethoprim-sulfamethoxazole, which is generally used as a first-line antibiotic in the management of uncomplicated UTIs. Similarly, a recent study conducted by [Kot, \(2019\)](#) proved that the UPEC resistance to fluoroquinolones in the developing countries is notably high (56-86 %). Moreover, results obtained by [Li et al., \(2017\)](#) demonstrated significant resistance (60.5 %) of *E. coli* to trimethoprim/ sulfamethoxazole, ampicillin, and cefazolin.

**Table 2.** Antibiotic sensitivity pattern of the 22 *Lactobacillus* isolates

<i>Lactobacillus</i> isolates	Antibiotics								
	AMP	KZ	TE	C	VA	E	CN	DA	CIP
L2	S	I	S	S	R	S	S	S	R
L3	S	S	S	S	R	S	S	S	S
L5	S	S	S	S	R	S	S	S	S
L6	S	S	S	S	R	S	R	S	R
L7	S	S	S	S	R	S	S	S	S
L10	S	S	S	S	R	S	S	S	S
L11	S	S	S	S	R	S	S	S	S
L16	S	S	S	S	R	S	I	S	S
L17	S	S	S	S	R	S	S	S	S
L18	S	S	S	S	R	S	S	S	R
L20	S	S	S	S	R	S	S	S	S
L23	S	S	S	S	R	R	R	S	S
L27	S	S	S	S	R	S	S	S	S
L28	S	S	S	S	R	S	S	S	S
L29	S	R	S	S	R	S	R	S	S
L30	S	S	S	S	R	R	R	S	S
L31	S	S	S	S	R	S	S	S	S
L33	R	S	S	S	R	S	S	S	R
L36	S	S	S	S	R	S	S	S	S
L37	S	S	S	S	R	S	S	S	S
L39	S	S	S	S	R	S	S	S	S
L40	S	S	S	S	R	S	R	S	R

Results are averages of three replicate plates. Where; S: sensitive, R: resistant, I: intermediate, AMP: ampicillin, KZ: cefazolin, TE: tetracycline, C: chloramphenicol, VA: vancomycin, E: erythromycin, CN: gentamicin, DA: clindamycin, CIP: ciprofloxacin

[Delcaru et al., \(2016\)](#) proved that microbial biofilms are responsible for the recurrences and relapses of UTIs. Biofilms that adhere on urinary catheters increase the percentage of nosocomial infections in hospitalized patients. In this study, 34% of *E. coli* isolates showed strong biofilm formation capability. In agreement, higher percentage was recorded by [Karigoudar et al., \(2019\)](#), as among the catheterized patients; 89.7 % of UPEC isolates were biofilm producer detected through the tissue culture plate method.

Thus, the search for new alternatives in antibacterial therapy is required, with a special interest in natural remedies as reported by [Silva et al., \(2019\)](#). In this context, [de Melo Pereira et al., \(2018\)](#) stated that

*Lactobacilli* are among the best-known probiotics. In our study, 41 (82 %) *Lactobacillus* isolates were recovered from stool samples collected from healthy breast-fed infants. Our results were confirmed by previous studies conducted by [Bahri et al., \(2014\)](#); [Davoodabadi et al., \(2015a, b\)](#) ; [Kaur et al., \(2018\)](#) that isolated probiotic *Lactobacillus* species from the faecal samples of healthy infants. Probiotic *Lactobacillus* isolates from human intestines are more useful than those from other origins, as they are highly resistant to elevated concentrations of bile salts and low pH levels. In addition, their adherence ability is superior compared to probiotics isolated from dairy products, and can be consumed safely in case of lactose intolerance, as proved by [Davoodabadi et al., \(2015a\)](#); [Sornplang and Piyadeatsoontorn, \(2016\)](#).

In the present work, the antibacterial potency of 41 CFSs of *Lactobacillus* isolates against 5 MDR *E. coli* isolates was investigated by disk diffusion method. We found that all the tested CFSs were able to inhibit the growth of the MDR and strong biofilm forming *E. coli* isolates. This is consistent with the previous results obtained by [Ezeonu and Kanu, \(2016\)](#) that demonstrated the inhibitory potential of *L. acidophilus* CFS against *Staphylococcus aureus*, *E. coli* and *Klebsiella* sp. in urine. Furthermore, current results were confirmed by [Davoodabadi et al., \(2015b\)](#) study, who proved that probiotic *L. fermentum*, *L. paracasei* and *L. plantarum* collected from faecal samples of healthy infants inhibited the growth of bacterial enteropathogens. The antibacterial properties of the probiotics CSFs were attributed to the production of organic acids that lowered the pH, in addition to the bioactive compounds released by these probiotics such as; bacteriocins and hydrogen peroxide, as confirmed by [Yang et al., \(2014\)](#). In the present work, the antibiofilm activity of 22 CFSs of *Lactobacillus* isolates was investigated using the microtitre plate assay. It was found that 16 CFSs exhibited more than 50 % reduction in the biofilm formed by the tested MDR *E. coli* isolates. *Lactobacillus* isolates L3, L7, L28 and L31 showed more than 80% reduction of biofilm formed by some *E. coli* isolates. In addition, the SEM micrographs showed significant changes in the structure of biofilms formed by the strong biofilm producer MDR *E. coli* isolate (E17), when co-cultured with some *Lactobacillus* isolates. As demonstrated by [Terraf et al., \(2012\)](#), biofilm formation by Lactobacilli could enhance colonization and is more permanent in the host mucosa, thus it inhibits colonization by some pathogenic strains. In accordance, [Abdelhamid et al., \(2018\)](#) proved that *L. helveticus* CFS grown in skim milk inhibited the biofilms formed by MDR *E. coli* isolate by 69.49 %, while CSF obtained from *L. plantarum* showed 64.57% reduction. They illustrated that this antibiofilm action was attributed to the antibacterial bioactive compounds. Also, [Osama et al., \(2017\)](#) demonstrated the antibiofilm potential of some *L. rhamnosus* and *L. gasseri* strains against *E. coli*,

*Pseudomonas aeruginosa*, and *S. aureus*. [Fernandes et al., \(2019\)](#) explained that the antibacterial substances such as; organic acids, hydrogen peroxide, bio-surfactants as well as bacteriocins, are responsible for this antibiofilm action. They also proved that this activity is strain-specific and may not appear in all *Lactobacillus* strains. [Davoodabadi et al., \(2015b\)](#) highlighted that the principle characteristics for choosing bacterial spp. as beneficial probiotics are their acid and bile salt tolerance, antibiotic sensitivity, and antimicrobial action against pathogenic microorganisms. Acid tolerance at standard pH (3) and bile salt tolerance at bile salt concentration 0.3, which resemble the normal level of human intestine, are important for characterization of probiotic *Lactobacillus* strains, as reported by [Halder et al., \(2017\)](#). Our results revealed that all isolated Lactobacilli were resistant to 2% bile salt and grow normally pH 3. These results proved that Lactobacilli isolated in this work can tolerate unfavorable conditions including growth at high bile salts or low pH *in vitro*, which are prerequisite probiotic characteristics that should be further studied *in vivo*.

Probiotic Lactobacilli must be safe; as they should not cause hemolysis in host body cells. Besides, they should be antibiotic sensitive, in order not to transfer resistance to the other intestinal pathogenic bacteria. However, [Halder et al., \(2017\)](#) reported that probiotic bacteria with antibiotic resistance that is not transferred or innate, is acceptable for formulation as products that can be safely consumed by human. In this study, *Lactobacillus* isolates showed high sensitivity to tetracycline; chloramphenicol, and clindamycin, in concurrence with the previous reports of [Maragkoudakis et al., \(2006\)](#); [Wang et al., \(2010\)](#). Currently, some *Lactobacillus* isolates showed resistance to several antibiotics including; erythromycin, gentamicin and ciprofloxacin; in addition to their resistance to vancomycin. Previous studies of [Verdenelli et al., \(2009\)](#); [Morrow et al., \(2012\)](#) attributed this result to the fact that Genus *Lactobacillus* is naturally resistant to vancomycin, and genes of such resistance are encoded on chromosomes;

thus they could not be induced or transferred to the other bacterial species. Knowing that antibiotic resistance may be a potential alert; thus the beneficial probiotic strain must be eliminated or not utilized once it acquires and/or becomes a reservoir of these genes. Finally, antibiotic resistance of *Lactobacillus* probiotic strains can be helpful only in case of individuals with unbalanced gut microbiota, which is attributed to the misuse and abuse of different antibiotics. Otherwise, the *Lactobacillus* resistance genes can be transferred to other intestinal microorganisms, as revealed by [Imperial and Ibana, \(2016\)](#).

The current study identified *Lactobacillus* isolates predominantly as; *L. acidophilus*, *L. plantarum*, *L. fermentum* and *L. paracasei*. In consistent with results of this study, [Mirlohi et al., \(2008\)](#) reported that *L. acidophilus* as the most predominant *Lactobacillus* isolate, followed by *L. plantarum*. On the contrary, [Davoodabadi et al., \(2015a\)](#) demonstrated that *L. fermentum* was the most frequent isolate recovered from infants stool samples, followed by *L. plantarum* and then *L. rhamnosus*.

## Conclusion

Based on observations of the present work, we concluded that *Lactobacillus* strains with great probiotic potential can be recovered from faeces of healthy infants. Some Lactobacilli supernatants have both antibacterial and antibiofilm potential toward multi-drug resistant uropathogenic *E. coli*. This supports their use in the prevention of bacterial UTI, and their future application as antibacterial alternatives against MDR and biofilm forming *E. coli* strains. Further *in vitro* and *in vivo* studies on these probiotic strains are still required.

## Conflict of interest

The authors declare no conflict of interests.

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This work did not receive fund from any profit or non-profit organization.

## Ethical approval

The protocols used in the study were approved by the Ethics Committee of the Minia University Hospital, El-Minia, Egypt. The patient's consents and statement of protection of the patient's privacy are provided.

## 5. References

- Abdelhamid, A.G.; Esaam, A. and Hazaa, M.M. (2018).** Cell free preparations of probiotics exerted antibacterial and antibiofilm activities against multidrug resistant *E. coli*. Saudi Pharmaceutical Journal. 26(5): 603-607.
- Ahrné, S.; Lönnemark, E.; Wold, A.E.; Aberg, N.; Hesselmar, B.; Saalman, R.; Strannegård, I.L.; Molin, G. and Adlerberth, I. (2005).** Lactobacilli in the intestinal microbiota of Swedish infants. Microbes and Infection. 7(11-12): 1256-62.
- Ali, I.; Rafaque, S.; Ahmed, S.; Malik, J.I. and Dasti, P. (2016).** Prevalence of multi-drug resistant uropathogenic *Escherichia coli* in Potohar region of Pakistan. Asian Pacific Journal Tropical Biomedicine. 6(1): 60-6.
- Aly, S.E.; Abo-Sereih, N.A.; Salim, R.G. and Hathout, A.S. (2018).** Isolation and Molecular Identification of Food Grade Lactic Acid Bacteria and Their Antifungal Activity. Journal of Biological Sciences. 18(6): 260-269.
- Aslam, B.; Wang, W.; Arshad, M.I.; Khurshid, M.; Muzammil, S.; Rasool, M.H.; Nisar, M.A.; Alvi, R.F.; Aslam, M.A.; Qamar, M.U.; Salamat, M. and Baloch, Z. (2018).** Antibiotic resistance: a rundown of a global crisis. Infection and Drug Resistance. 11: 1645-1658.
- Bahri, F.; Lejeune, A.; Dubois-Dauphin, R.; Elmejdoub, T.; Boulahrouf, A. and Thonart, P. (2014).** Characterization of *Lactobacillus* strains isolated from Algerian children faeces for their

probiotic properties. African Journal of Microbiology Research. 8(3): 297-303.

**Borchert, D.; Sheridan, L.; Papatsoris, A.; Faruqz, Z.; Barua, J.M.; Junaid, I.; Pati, Y.; Chinegwundoh, F. and Buchholz, N. (2008).** Prevention and treatment of urinary tract infection with probiotics: Review and research perspective. Indian Journal of Urology. 24: 139-144.

**Cheesbrough, M. (1981).** Medical Laboratory Manual for Tropical Countries. In Cheesbrough, M. (Editor). Vol. (1). Cambridge University press, UK.

**Davoodabadi, A.; Dallal, M.M.S.; Lashani, E. and Ebrahimi, M.T. (2015a).** Antimicrobial Activity of *Lactobacillus* spp. Isolated From Fecal Flora of Healthy Breast-Fed Infants Against Diarrheagenic *Escherichia coli*. Jundishapur Journal of Microbiology. 8(12): e27852.

**Davoodabadi, A.; Dallal, M.M.S.; Foroushani, A.R., Douraghi, M.; Yazdi, M.K.S. and Harati, A.F. (2015b).** Antibacterial activity of *Lactobacillus* spp. isolated from the feces of healthy infants against enteropathogenic bacteria. Anaerobe. 34: 53-8.

**de Melo Pereira, G.V.; de Oliveira Coelho, B.; Júnior, A.I.M.; Thomaz-Soccol, V. and Soccol, C. R. (2018).** How to select a probiotic? A review and update of methods and criteria. Biotechnology Advances. 36(8): 2060-2076.

**Delcaru, C.; Alexandru, I.; Podgoreanu, P.; Grosu, M.; Stavropoulos, E.; Chifiriuc, M. C. and Lazar, V. (2016).** Microbial Biofilms in Urinary Tract Infections and Prostatitis: Etiology, Pathogenicity, and Combating strategies. Pathogens. 5(4): 65.

**Donlan, R.M. (2001).** Biofilms and device-associated infections, Emerging Infectious Diseases Journal. 7: 277-281.

**Eberly, A.R.; Floyd, K.A.; Beebout, C.J.; Colling, S.J.; Fitzgerald, M.J.; Stratton, C.W.; Schmitz, J.E. and Hadjifrangiskou, M. (2017).** Biofilm Formation by Uropathogenic *Escherichia coli* Is Favored under

Oxygen Conditions That Mimic the Bladder Environment. International Journal of Molecular Sciences. 18(10): 2077.

**Emody, L.; Kerenyi, M. and Nagy, G. (2003).** Virulence factors of uropathogenic *Escherichia coli*. International Journal of Antimicrobial Agents. 22(2): 29-33.

**Ezeonu, I.M. and Kanu, J.U. (2016).** Inhibition of biofilms on urinary catheters using immobilized *Lactobacillus* cells. African Journal of Microbiology Research. 10(25): 920-929.

**FAO/WHO. (2002).** Food and Agriculture Organization/ World Health Organization. "Guidelines for the evaluation of probiotics in food, Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food".

**Fernandes, M.S.M.; Lourenço, M.L.M.C.; Vasconcelos, B.M. and Carneiro, V.A. (2019).** Probiotics *Lactobacillus* strains: A promising alternative therapy against to biofilm-forming enteropathogenic bacteria?. African Journal of Microbiology Research. 13(28): 544-551.

**Halder, D.; Mandal, M.; Chatterjee, S.S.; Pal, N.K. and Mandal, S. (2017).** Indigenous Probiotic *Lactobacillus* Isolates Presenting Antibiotic like Activity against Human Pathogenic Bacteria. Biomedicines. 5(31): 1-11.

**Hudzicki, J. (2009).** Kirby-Bauer disk diffusion susceptibility test protocol. American Society of Microbiology. pp. 1-20. <https://www.asm.org/getattachment/2594ce26-bd4447f6-8287-0657aa9185ad/>

**Imperial, I.C. and Ibane, J.A. (2016).** Addressing the Antibiotic Resistance Problem with Probiotics: Reducing the Risk of Its Double-Edged Sword Effect. Frontiers in Microbiology. 7(1983): 1-10.

**Kacem, M. and Kaid-Harche, M. (2008).** Probiotic characteristics of *Lactobacillus plantarum* strains from

traditional butter made from camel milk in arid regions (Sahara) of Algeria. *Grasas y Aceites*. 59: 216-222.

**Karigoudar, R.M., Karigoudar, M.H.; Wavare, S.M., and Mangalgi, S.S. (2019).** Detection of biofilm among uropathogenic *Escherichia coli* and its correlation with antibiotic resistance pattern. *Journal of Laboratory Physicians*.11(1): 17-22.

**Kaur, S.; Sharma, P.; Kalia, N.; Singh, J. and Kaur, S. (2018).** Anti-biofilm Properties of the Fecal Probiotic Lactobacilli Against *Vibrio* spp. *Frontiers in Cellular and Infection Microbiology*. 8(120): 1-14.

**Kiliç, G.B. and Karahan, A.G. (2010).** Identification of lactic acid bacteria isolated from the fecal samples of healthy humans and patients with dyspepsia, and determination of their pH, bile, and antibiotic tolerance properties. *Journal of Molecular Microbiology and Biotechnology*. 18(4): 220-229.

**Kot, B. (2019).** Antibiotic Resistance Among Uropathogenic *Escherichia coli*. *Polish Journal of Microbiology*. 68(4): 403-415.

**Lebeer, S.; Vanderleyden, J. and De Keersmaecker, S.C.J. (2008).** Genes and molecules of lactobacilli supporting probiotic action. *Microbiology and Molecular Biology Reviews*. 72: 728-764.

**Li, X.F.; Liu, Z.J.; Chen, X.; Li, J.; Cui, Z.G.; Kan, B.; Ma, J.R. and Cui J.H. (2017).** Study on antibiotic resistance of *Escherichia coli* and *Enterococcus* colonized in intestine of neonates from neonatal intensive care unit. *Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi*. 38: 1259-1262.

**Maragkoudakis, P.A.; Zoumpoulou, G.; Miaris, C.; Kalantzopoulos, G.; Pot, B. and Tsakalidou E. (2006).** Probiotic potential of *Lactobacillus* strains isolated from dairy products. *International Dairy Journal*. 16: 189-199.

**Mirlohi, M.; Soleimani-Zad, S. and Sheikh-Zeinodin, M. (2008).** Identification of Lactobacilli from Fecal Flora of Some Iranian Infants. *Iranian Journal of Pediatrics*. 18(4): 357-363.

**Morrow, L.E.; Gogineni, V., and Malesker, M.A. (2012).** Probiotics in the intensive care unit. *Nutrition in Clinical Practice*. 27(2): 235–241.

**Nouri, M.; Rahbarizadeh, F.; Ahmadvand, D.; Moosakhani, F.; Sadeqzadeh, E.; Lavasani, S. and Vishteh, V.K. (2010).** Inhibitory effects of *Lactobacillus salivarius* and *Lactobacillus crispatus* isolated from chicken gastrointestinal tract on *Salmonella enteritidis* and *Escherichia coli* growth. *Iranian Journal of Biotechnology*. 8: 32-37.

**Osama, D.M.; Elkhatib, W.F.; Tawfeik, A.M. and Mohammad, M. (2017).** Antimicrobial, antibiofilm and immunomodulatory activities of *Lactobacillus rhamnosus* and *Lactobacillus gasseri* against some bacterial pathogens. *International Journal of Biotechnology for Wellness Industries*. 6(1): 12-21.

**Ozgun, D. and Vural, H. (2011).** Identification of *Lactobacillus* strains isolated from faecal specimens of babies and human milk colostrum by API 50 CHL system. *Journal of Medical Genetics and Genomics*. 3(3): 46-49.

**Ramírez-Castillo, F.Y.; Moreno-Flores, A.C.; Avelar-González, F.J.; Márquez-Díaz, F.; Harel, J. and Guerrero-Barrera, A.L. (2018).** An evaluation of multidrug-resistant *Escherichia coli* isolates in urinary tract infections from Aguascalientes, Mexico: cross-sectional study. *Annals of Clinical Microbiology and Antimicrobials*. 17(1): 1-13.

**Rinttilä, T.; Kassinen, A.; Malinen, E.; Krogius, L. and Palva, A. (2004).** Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. *Journal of Applied Microbiology*. 97: 1166-1177.

**Sambanthamoorthy, K.; Feng, X.; Patel, R.; Patel, S. and Parnavitana, C. (2014).** Antimicrobial and antibiofilm potential of biosurfactants isolated from lactobacilli against multi-drug-resistant pathogens. *BMC Microbiology*. 14(1): 1-9.

- Silva, D.R.; Rosalen, P.L.; Freires, I.A.; Sardi, J.; Lima, R.F.; Lazarini, J.G.; Costa, T.; Pereira J.V.; Godoy, G.P. and Costa, E. (2019).** *Anadenanthera Colubrina* vell Brenan: anti-Candida and antibiofilm activities, toxicity and therapeutical action. Brazilian Oral Research. 33: 1-11.
- Sornplang, P. and Piyadeatsoontorn, S. (2016).** Probiotic isolates from unconventional sources: A review. Journal of Animal Science and Technology. 58(26): 1-11.
- Stepanović, S.; Vuković, D.; Hola, V.; Di Bonaventura, G.; Djukić, S.; Cirković, I., and Ruzicka, F. (2007).** Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica. 115(8): 891-899.
- Terraf, M.L.; Tomás, M.S.J.; Nader-Macías, M.E.F. and Silva, C. (2012).** Screening of biofilm formation by beneficial vaginal Lactobacilli and influence of culture media components. Journal of Applied Microbiology. 113(6): 1517-1529.
- Toval, F.; Köhler, C.D.; Vogel, U.; Wagenlehner, F.; Mellmann, A.; Fruth, A.; Schmidt, M.A.; Karch, H.; Bielaszewska, M. and Dobrindt, U. (2014).** Characterization of *Escherichia coli* isolates from hospital inpatients or outpatients with urinary tract infection. Journal of Clinical Microbiology. 52(2): 407-418.
- Verdenelli, M.C.; Ghelfi, F.; Silvi, S.; Orpianesi, C.; Cecchini, C. and Cresci, A. (2009).** Probiotic properties of *Lactobacillus rhamnosus* and *Lactobacillus paracasei* isolated from human faeces. European Journal of Nutrition. 48(6): 355-363.
- Wadoum, R.E.G.; Florence, F.A.; Marie, K.P.; Kamdem, U.L.B.; Kenfack, C.H.; Edith-Marius, F.K.; Nzekwa, N.; Horpa, E.; Colizzi, V. and Ngoufack, F.Z. (2019).** *In Vitro* Antimicrobial Characterization of *Lactobacillus* Isolates Towards Their Use as Probiotic Alternatives to Antibiotic Growth Promoters. International Journal of Microbiology and Biotechnology. 4(3): 72-86.
- Wang, C.Y.; Lin, P.R.; Ng, C.C. and Shyu, Y.T. (2010).** Probiotic properties of *Lactobacillus* strains isolated from the faeces of breast-fed infants and Taiwanese pickled cabbage. Anaerobe. 16(6): 578-585.
- Wayne, P. (2018).** Performance Standards for Antimicrobial Susceptibility Testing. CLSI supplement M100. Clinical and Laboratory Standards Institute, 28<sup>th</sup> Edition.
- Wu, C.C.; Lin, C.T.; Wu, C.Y.; Peng, W.S.; Lee, M.J. and Tsai, Y.C. (2015).** Inhibitory effect of *Lactobacillus salivarius* on *Streptococcus mutans* biofilm formation. Molecular oral Microbiology. 30: 16-26.
- Yang, S.C.; Lin, C.H.; Sung, C.T. and Fang, J.Y. (2014).** Antibacterial activities of bacteriocins: application in foods and pharmaceuticals. Frontiers in Microbiology. 5(241):1-10.